Correlations between Selenium Levels and Diabetes Mellitus Type 2 in Chronic Hepatitis C-Infected Patients

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Abstract

Background: **Plasma selenium (Se) concentrations corresponding to optimal health are highly dynamic and based on a combination of factors that need to be considered when assessing epidemiological data, such as the findings linking serum Se and type 2 diabetes mellitus (DM). High serum Se concentrations may be associated with a higher occurrence of diabetes.** *Aim:* **To evaluate the correlations between serum Se levels and DM in patients with chronic Hepatitis C (CHC).** *Patients and Methods:* **This case-control study included three groups; group 1 (29 CHC patients with DM), group 2 (29 CHC patients without DM) and group 3 (29 healthy control). Subjects were tested for biochemical liver function tests, fasting blood sugar [FBS], and fasting insulin levels). Homeostasis model assessment (HOMA)-insulin resistance (IR) was calculated. Se levels were measured using Atomic Absorption Spectroscopy.** *Results:* **serum Se were significantly higher in group 1 and group 3 compared to group 2 (p<0.01). Meanwhile, serum Se were insignificantly higher in group 1 than in group 3 (p>0.05). Cutoff value to differentiate diabetic and non-diabetic CHC patients using serum Se was >10 µg/l with sensitivity (76%) and, specificity (86.2%). The incidence and the risk of Se deficiency were significantly higher among group 2 than among group 2 (24.1% *vs.* 3.4%, respectively) (p<0.05). In entire CHC patients (group 1+group 2), the mean HOMA-IR values were significantly lower in the group with deficient Se levels than in the group with normal Se levels (p=0.005). A negative correlations between serum Se levels and ALT levels, AST levels, and diabetes treatment was found.** *Conclusions:* **Serum Se levels were significantly higher in CHC+DM group and in control group than in CHC group. The incidence and the risk of Se deficiency were higher among HCV group compared to CHC+DM group.**

Keywords: Selenium, Diabetes Mellitus, Hepatitis C

Introduction

Hepatitis C Virus (HCV) infection is considered a major and serious health-problem in Egypt. It is considered a frequent cause of CHC, and leads to the development of cirrhosis and hepatocellular carcinoma (HCC). It is estimated that about 150 to 200 million people are infected with HCV worldwide, and approximately 85% of them will be chronically infected[1]. DM is one of the most common endocrine diseases, and it can

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lead to kidney failure, blindness, nerve damage, amputation. Also DM is a major risk factor for atherosclerosis, hypertension, stroke, heart disease, birth defects, all ultimately associated with increased mortality risk. DM type 2 is the most prevalent pattern of diabetes, currently affecting more than 300 million people worldwide[2]. It is characterized by the combination of IR and pancreatic β cell function failure[3, 4]. It has long be known that glucose intolerance is a common complication of most chronic liver diseases, independently of the etiology, especially at the advanced stages. But, studies had shown that CHC patients are associated with an increased risk of developing IR greater than patients with other types of chronic liver disease. This risk is at least 2 folds as compared to both general population and people with other liver disease, and up to one third of HCV patients develop DM type 2[5]. Se is one of the essential trace elements. Human needs just a bit of it for normal cellular functioning, but it can be toxic if taken too much[6, 7]. The average daily need is about 55 μg, however, it becomes harmful when the dose exceeds 400 μg/d (Selenosis). Plasma concentration of Se is the best indicator of Se status, plasma seleno-protein P and plasma glutatione perioxidase activity are considered also biomarkers to assess Se status[8]. Se is vital for normal glucose metabolism. Plasma Se concentrations corresponding to optimal health are highly dynamic and based on a combination of factors that need to be considered when assessing epidemiological data, such as the findings linking serum Se and type 2 DM. High serum Se concentrations, which reflect dietary intake, are associated with a higher oc-

currence of diabetes, higher FBS and increased glycosylated hemoglobin levels[9,10]. This hypothesis suggests that Se levels associated with the severity of hepatic fibrosis in patients with CHC is likely to be one of the factors contributing to IR in those patients[11]. Diabetic patients should avoid Se supplements until randomized controlled trials show objective benefits on mortality or morbidity end points[12].

Patients and Methods

This case-control study was conducted to compare the serum level of Se in chronic HCV patients with and without type 2 diabetes. It was performed at Clinical Pathology and Internal Medicine Departments, Suez Canal University hospital.

Subjects
Three groups were enrolled; the first group included 29 CHC patients with DM, the second group included 29 CHC patients without DM and the third group included 29 healthy blood donors (anti-HCV negative and non-diabetic). The age of the studied subjects ranged from 24-60 years, both genders were included. CHC infection defined as positive anti-HCV antibodies and/or positive HCV-RNA for more than 6 months[13]. Staging of the severity of chronic liver disease were from A to C according to Child-Pugh score[14]. Definite diagnosis of DM was performed according to World Health Organization (WHO) criteria where patient was considered diabetic if he has a single raised glucose reading (FBS ≥126mg/dl, PPS ≥140 mg/dl, RBS ≥199.8mg/dl and hemoglobin A1c ≥6.9%) with symptoms, or raised values on two occasions (FBS and 2 hours PPS) even if there is no symp-

toms, or raised RBS only but with typical symptoms (poly-depsia, polyuria, or hunger)[15,16]. Patients with positive hepatitis B markers, HIV and HCC were excluded. Patients known to be diabetic before the development of chronic HCV infection were also excluded.

Methods

All patients were subjected to baseline assessment for each case by history through interview checklist question-naire including; personal history (age and gender), past history of any chronic illnesses (DM, hypertension or renal failure). Full medical history of multivitamin/mineral supplements, vitamin B complex, calcium supplementation, glucosamine sulfate, insulin/oral hypoglycemic drugs and anti-hypertensive drugs and Family history of DM. Anthropometric measurements including body mass index (BMI) was calculated as weight (kg) / height (m²).

Table 1: Demographic and clinical characteristics of the studied populations

Variables	Group 1 (n=29)	Group 2 (n=29)	Group 3 (n=29)	p-value
Age (years)				
Mean ±SD	44.5±8.3	41.3±8.3	39.1±8.9	0.7
Range	30-60	25-56	24-55	
Gender				
Male	10 (34.5%)	14 (48.3%)	15 (51.7%)	0.42
Female	19 (65.5%)	15 (51.7%)	14 (48.3%)	
BMI (kg/m²)				
Mean ±SD	20.6±2.99	21.3±2.4	19.7±2.1	0.009**
Range	16-25	17-25	16-24	
SBP (mmHg)				
Mean ±SD	114.7±21.4	111.7±18.1	110.3±13.4	0.64
Range	80-160	70-140	80-130	
DBP (mmHg)				
Mean ±SD	81.6±15.7	76.2±11.1	77.8±6.9	0.21
Range	60-120	50-95	65-90	
DM treatment				
Yes	16 (55.2%)	-	-	-
No	13 (44.8%)	-	-	

*Significant p-value ≤0.05, **highly significant p-value ≤0.01, SBP= systolic blood pressure, DBP= diastolic blood pressure, DM=diabetes mellitus.

About 5 ml of blood was withdrawn from each patient by the researcher. Blood was collected into labeled vacuum tube, centrifuged at 3000 g for 10 min, and serum was separated in eppendorf and stored frozen at -20°C till analysis. The following biochemical parameters were done for every patient; liver function tests: ALT, AST, GGT, albumin, bilirubin and PT. FBS levels after 8-12 hours fasting and fasting insulin level were performed. HOMA-IR was calculated as (fasting insulin μ/L x FBS mg/dl) /405 or (fasting insulin mu/ml x FBS mmol/L) / 22.5. All biochemical parameters were performed using fully automated auto-analyzer Cobas C501 (Roche diagnostics, Germa-

ny) . Assessment of serum selenium level was done using Atomic Absorption Spectroscopy. Light of a specific wavelength is passed through the atomic vapor of an element of interest, and measurement is made of the at-tenuation of the intensity of the light as a result of the absorption. Selenium will absorb ultraviolet light in its elemental form when it is excited by heat, either by flame or graphite furnace[17].

Table 2: Liver function tests of the studied populations

Variables	Group 1 (n=29)	Group 2 (n=29)	Group 3 (n=29)	p-value
AST (U/ml) Mean ±SD Range	52.7±13.1 36-85	50.97±19.8 13-96	26.3±5.7 18-39	<0.0001**
ALT (U/ml) Mean ±SD Range	36.3±8.8 22-57	35.6±14.5 8-60	17.03±4.3 11-28	<0.0001**
GGT (IU/L) Mean ±SD Range	25.2±6.2 17-41	23.8±4.8 17-35	19.5±4.7 10-27	<0.0001**
PT Mean ±SD Range	16.0±4.4 13-32	15.4±3.0 13-25	12.8±0.1 12.5-13.0	<0.0001**
Albumin (gm/dl) Mean ±SD Range	2.9±0.99 1.3-4.9	3.5±0.89 1.8-5.0	4.2±0.60 3.0-5.0	<0.0001**
T. bilirubin (mg/dl) Mean ±SD Range	2.3±1.2 1-4	1.5±1.02 0.1-4	0.76±0.44 0.1-1	<0.0001**

**highly significant p-value ≤0.01, ALT= Alanine transaminase, AST= Aspartate transaminase, GGT= Gamma-glutamyl transpeptidase, PT= Prothrombin time.

Results

The mean age and gender of the studied groups were comparable (p=0.7 and p=0.42 respectively). The mean values of BMI were higher in the first group (chronic HCV patients with DM) and the second group (chronic HCV patients without DM) than in the third group (healthy control) (20.6 kg/m^2 *vs.* 21.3 kg/m^2 *vs.* 19.7 kg/m^2, respectively) (p=0.009). There were no statistically significant differences between the studied groups regarding mean SBP and DBP (p=0.64 and 0.21, respectively). In the first group, 55.2% of the pa tients administered treatment for diabetes (Table 1). The mean values of AST, ALT, GGT, PT and total bilirubin were significantly higher in the first and the second groups than in the third group, while the mean values of serum albumin were significantly lower in the 1st and the 2nd groups than in the 3rd group (p<0.0001). Moreover, the mean values of total bilirubin were significantly higher in the 1st group than in the 2nd group (p<0.0001), while the mean serum albumin were significantly lower in the first group than in the second group (<0.0001) (Table 2).

Table 3: Incidence of Se deficiency among the studied populations

Variables	Group 1 (n=29)	Group 2 (n=29)	Group 3 (n=29)	OR	95% CI	p-value
Se levels Deficient (≤5 µg/l) Normal (>5 µg/l)	1 (3.4%) 28 (96.6%)	7 (24.1%) 22 (75.9%)	0 29 (100%)	8.9	1.0-78.0	0.02*

*Significant p-value ≤0.05, **highly significant p-value ≤0.01, OR= odds ratio, CI= confident interval.*

Table 4: Incidence of insulin resistance (IR) among the studied populations

Variables	Group 1 (n=29)	p1	Group 2 (n=29)	p2	Group 3 (n=29)	p3
IR IR (≥2) No IR (<2)	24 (82.8%) 5 (17.2%)	0.003**	12 (41.4%) 17 (58.6%)	0.043*	5 (17.2%) 24 (82.8%)	<0.0001**

**highly significant p-value ≤0.01, p1=p-value between group1 and 2, p2=p-value between group2 and 3, p3=p-value between group1 and 3.

The mean values of FBS, fasting insulin and HOMA-IR were significantly higher in the first than in the second group and the third group (p<0.0001). The mean values of fasting insulin and HOMA-IR were significantly higher in the second group than in the third group (p<0.0001). The mean values of serum Se were significantly higher in the first group and in the third group in comparison to the second group (p<0.0001). Meanwhile, the mean values of serum Se were insignificantly higher in the first group than in the third group (p=0.58) (Chart 1). The receiver operating characteristic (ROC) curve showed that the optimal cutoff value to differentiate diabetic and non-diabetic HCV patients using serum Se was >10 µg/l. Although the sensitivity of serum Se was relatively low (76%), it was more specific (86.2%) and had much better positive and negative predictive values (85% and 78.1%, respectively). The ROC curve for cutoff points of serum Se for detecting of type-2 diabetes mellitus in chronic HCV patients showed that serum Se more than 10

µg/l was the most accurate diagnostic value for detection of diabetes mellitus with an area under curve of (0.87) with significant level of (p<0.0001) (Chart 2). The patients were subdivided into normal serum Se (>5 µg/l) and deficient serum Se (≤5 µg/l). The incidence and the OR of Se deficiency were significantly higher among the second group than among the first group (24.1% versus 3.4%, respectively) (OR=8.9, 95% CI =1.0-78.0, p=0.026) (Table 3). In entire HCV patients (n=58), the mean HOMA-IR values were significantly lower in the group with deficient Se levels than in the group with normal Se levels (mean HOMA-IR were 2.3 µg/l versus 6.4 µg/l, respectively) (p=0.005). The incidence IR was significantly higher among the first group than among the second group (82.8% versus 41.4%, respectively) (p=0.003). The incidence IR was also significantly higher among the second group than among the third group (41.4% vs. 17.2%, respectively) (p=0.04) (Table 4). There were significant negative correlations between serum Se levels and ALT levels (r=-0.21, p=0.049),

AST levels (r=-0.22, p=0.048), and administered diabetes treatment (r=-0.36, p=0.043) (Table 5 and Charts 3-4). The correlations between serum Se levels and age, gender, BMI, SBP, DBP, FBS, fasting insulin, total bilirubin, serum albumin, PT, GGT, and HOMA-IR were statistically insignificant (p>0.05).

Table 5: Correlations between serum Se and the studied variables

	Pearson Correlation	p-value
Age	0.041	0.709
Gender	0.029	0.789
BMI	-0.098	0.365
SBP	0.036	0.739
DBP	0.072	0.509
FBS (mg/dl)	0.169	0.118
Fasting insulin (u/ml)	-0.053	0.624
Total bilirubin (mg/dl)	0.075	0.492
Serum albumin (gm/dl)	-0.054	0.622
PT	0.013	0.906
GGT (IU/L)	-0.032	0.768
ALT (U/ml)	-0.210	0.049*
AST (U/ml)	-0.220	0.048*
HOMA- IR	0.071	0.514
Diabetes treatment	-0.362	0.043*

Significant p-value ≤0.05.

Discussion

The essential trace mineral, Se, is of fundamental importance to human health. As a constituent of selenoproteins, Se has structural and enzymatic roles, in the latter context being best-known as an antioxidant and catalyst for the production of active hormones. Se is needed for the proper functioning of the immune system, and appears to be a key nutrient in counter acting the development of virulence and inhibiting virus progression. It is required for sperm motility and may reduce the risk of miscarriage. Se deficiency has been linked to adverse mood states. An elevated Se intake may be associated with reduced cancer risk. Findings have been equivocal in linking Se to cardiovascular disease risk although other conditions involving oxidative stress and inflammation have shown benefits of a higher Se status[18]. Plasma Se concentrations corresponding to optimal health are highly dynamic and based on a combination of factors that need to be considered when assessing epidemiological data, such as the findings linking serum Se and type 2 DM. High serum Se concentrations, which reflect dietary intake, are associated with a higher occurrence of DM, higher FBS and increased HBA1c [9,19-20].
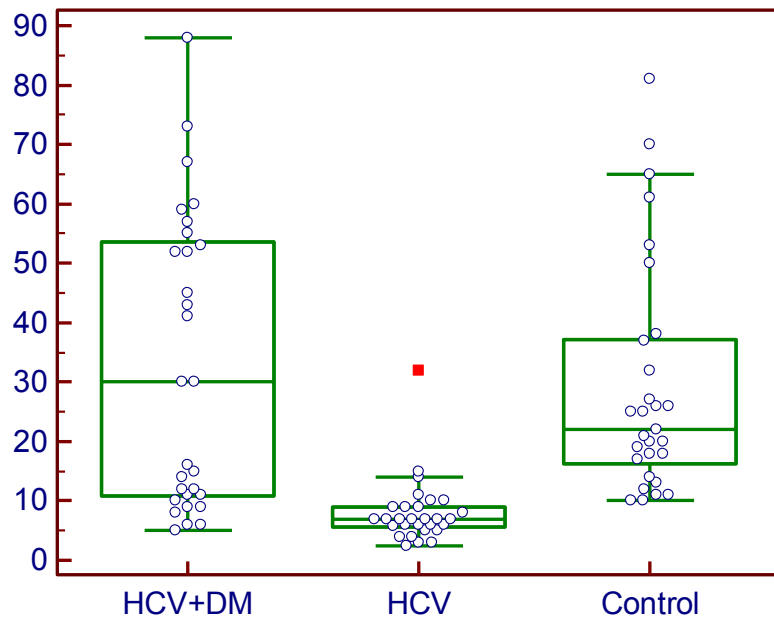
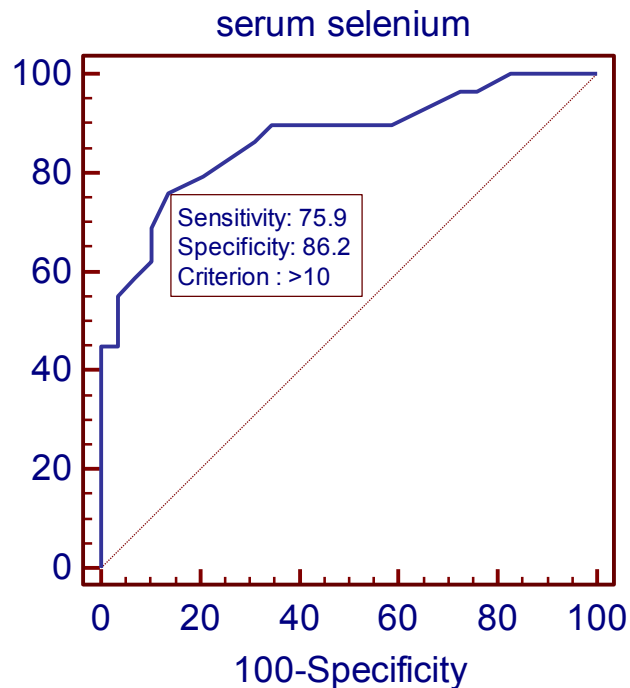**Figure 1:** Mean levels of serum Se among the studied populations

Figure 2: Receiver operating characteristic (ROC) curve with optimal cutoff value for detecting type-2 diabetes mellitus in chronic HCV patients.

In our study, the mean age was 44.5 years, 41.3 years and 39.1 years in the 1st, the 2nd and the 3rd groups, respectively. The studied groups were age-matched without any statistically significant differences (p>0.05). This comes in agreement with the results of Lehman and Wilson[21] who estimated the preva-

lence and the incidence of HCV infection in Egypt. They reported that the age group 40–44 years has very high prevalence rate of HCV infection (33%) with a mean annual incidence of 25.5 per 1000 population. Regarding gender, the frequencies of female patients were slightly higher than male patients in the first (HCV+DM) group and the second (HCV) group, but the differences between groups were insignificant (p<0.05). Similar results about gender differences were found in African study performed by Ayele and Gebre-Selassie[22]. Several previous Egyptian researches have reflected these different values between different genders[21,23-25]. Our data observed that the mean values of BMI were higher in the first (HCV+DM) and the second (HCV) groups than in the third group (healthy control) (20.6 kg/m^2 versus 21.3 kg/m^2 versus 19.7 kg/m^2, respectively) (p<0.01). In the same manner In agreement with this notification, Zhou et al.[26] found that the patients with type 2 DM had higher frequency of overweight and obesity than matched controls. Obesity (especially abdominal or visceral obesity) is one of the components of IR syndrome along with hyperinsulinemia, dyslipidemia of the high-triglyceride and/or low HDL type, and hypertension[27]. The current study indicated that the mean values of fasting insulin and HOMA-IR were significantly higher in the second (HCV) group than in the third (healthy control) group (p<0.01). Similar results were obtained by Muzzi et al.[28] and Taura et al. [29] who denoted that HCV infection is highly associated with IR. In agree-

ment with our study, a study performed by Lecube et al.[30] indicated that HCV-infected subjects had significant increase of the fasting insulin and significant decrease in insulin sensitivity. Furthermore, several studies suggested that IR promotes hepatic fibrosis. This hyper-insulinemia was suggested to have a major role in the development of hepatic fibrosis.[31,32]. The mean values of serum selenium were significantly higher in the first (HCV+DM) group and in the third (healthy control) group than in the second (HCV) group. Meanwhile, the mean values of serum Se were insignificantly higher in the first (HCV+DM) group than in the third (healthy control) group. Consistent with these results, Laclaustra et al.[19], Stranges et al.[9] and Rocourt and Cheng[33] reported that high serum Se concentrations are associated with a higher occurrence of diabetes, higher FBS and increased glycosylated hemoglobin levels. Epidemiological studies have shown an association between Se and type 2 DM. National health and nutrition examination survey (NHANES) indicated that serum Se is positively correlated with an increased incidence of type 2 DM[19,34]. In addition, another study conducted by Stranges et al.[35] indicated that individuals that had the highest baseline Se levels were at an increased risk for type 2 DM even when factors, such as age, sex, body mass index and smoking status were controlled. The incidence and the risk (OR=8.9) of Se deficiency were significantly higher among the second (HCV) group than among the first (HCV+DM) group (24.1% versus 3.4%, respectively).
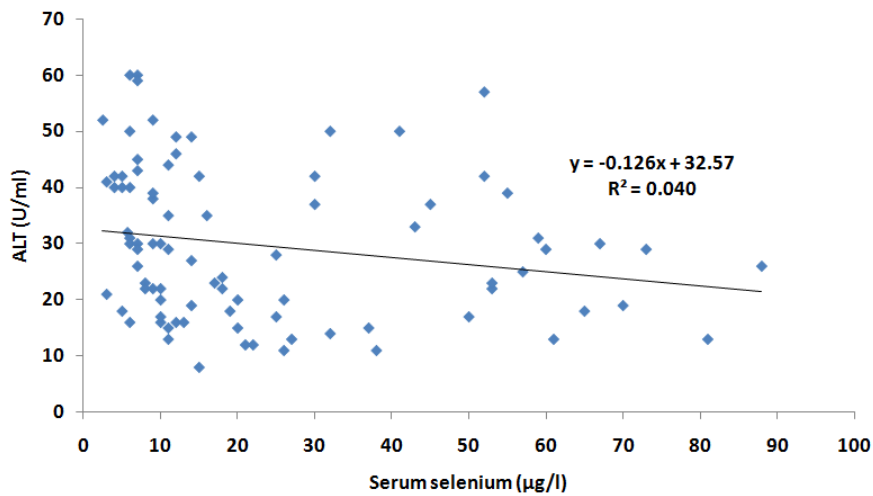
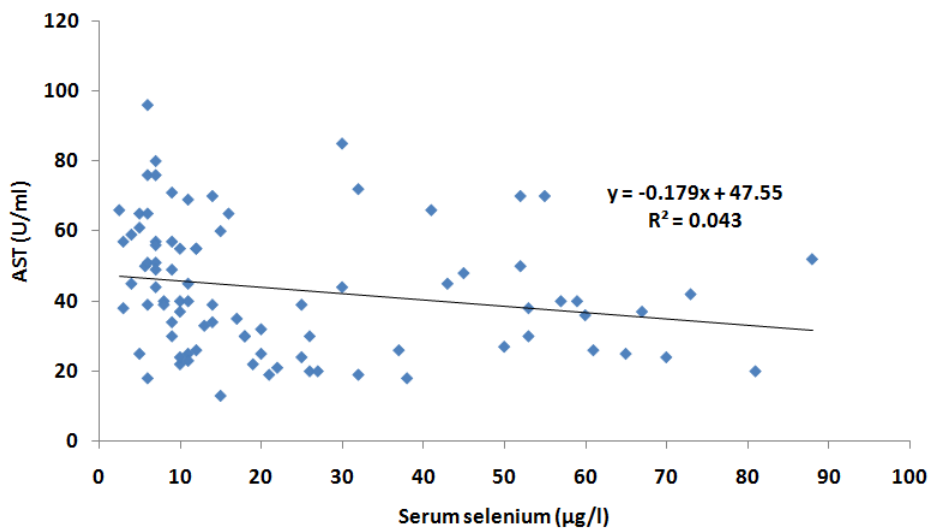**Figure 3:** Significant negative correlation between serum Se and ALT levels

$$y = -0.126x + 32.57$$
$$R^2 = 0.040$$

Figure 4: Significant negative correlation between serum Se and AST levels

$$y = -0.179x + 47.55$$
$$R^2 = 0.043$$

This can be explained by the fact, in HCV, the liver cells become affected with deterioration in its functions which cause this Se deficiency due to decease of selenoproteins biosynthesis. Meanwhile, dysregulation of carbohydrate metabolism in diabetes might affect serum Se levels, as the hepatic biosyn thesis of selenoproteins is suppressed by insulin and stimulated under hyperglycemic conditions. So, with higher blood sugar (hyperglycemia), the Se synthesis is increased, while, with administration of treatment (insulin), the Se synthesis is decreased. This was emphasized by our study as there was sig-

nificant negative correlations between serum Se levels and administered diabetes treatment (r=-0.36, p=0.043). In similar direction, low Se is a significantly greater risk factor (OR=16) in HCV patients, and conferred a more significant risk than deficiency of any other nutrient investigated. Low levels of Se were significantly and independently related to mortality and faster disease progression[36]. An assumed link between serum Se and type 2 IR/DM was discussed by Steinbrenner[37]. Se concentrations in the habitual diet and in dietary supplements are probably not sufficient to induce overt diabetes in healthy individuals. On the other hand, high serum Se and selenoprotein levels have been found to be associated with biomarkers of an impaired carbohydrate and lipid homeostasis in humans. Moreover, abundant expression of antioxidant selenoproteins due to dietary Se oversupply resulted in hyperinsulinemia and decreased insulin sensitivity in animal models. In HCV patients (group 1+group 2), the mean HOMA-IR values were significantly lower in the group with deficient Se levels than in the group with normal Se levels (2.3 µg/l versus 6.4 µg/l, respectively) (p=0.005). Rayman and Stranges [38] mentioned the findings from observational cross-sectional studies that high Se exposure is associated with type 2 diabetes or IR. Factors affecting serum Se are not just location and level of disease-associated inflammation, but the fact that higher concentrations of serum Se and lower concentrations of glutathione peroxidase are found in type 2 diabetic patients than in normal subjects. In conclusion, there is a clear association between higher serum Se and impaired glucose metabolism, IR and type 2 diabetes. In conclusion, this study found a close relationship between serum Se levels and DM in patients with chronic HCV which must be confirmed by further larger, more controlled studies.

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